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EFFECT OF COOLING METHOD AND INCUBATION TIME ON SURVIVAL OF BOAR SPERMATOZOA STORED IN THREE DIFFERENT COMMERCIAL EXTENDERS AT 5C

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Effect of cooling method and incubation time on survival of boar spermatozoa stored in three different commercial extenders at 5°C.

Project 20000059
RES: 70BX

Summary

Extended boar semen is stored at a temperature of 17-18°C as the sperm are extremely sensitive to chilling below 15°C. Storing liquid boar semen at 5°C would greatly improve the ability of Saskatchewan pork producers to transport semen without affecting its quality. The objectives were to determine the effects of stepwise cooling combined with different incubation times at 17°C on the ability to store liquid boar semen at 5°C using three commercial extenders. The results indicate it is possible to store liquid boar semen at 5°C with current extenders. Storing liquid boar semen at 5°C while maintaining adequate progressive motility, morphology and viability is possible provided the sperm are cooled in a direct manner to 17°C and incubated for 24 hr at 17°C prior to lowering the temperature to 5°C. This procedure however, does not maintain total motility values that are as great as those for sperm stored at 17°C. Whether or not this statistical difference is of practical significance will take an insemination trial to determine. Following successful validation, adoption of this protocol will allow Saskatchewan producers to transport and store boar semen at an easier temperature to maintain thus better protecting sperm from damage due to temperature fluctuations.

Technical Report

Background

During the last decade, an increasingly important technology in optimizing swine production systems has been the widespread adoption of artificial insemination. Large geographical distances often exist between production units in Saskatchewan and their source of boar semen, or between commercial boar studs in Saskatchewan and their customers. This makes shipping time lengthy which exposes the shipment to long-duration temperature challenges that vary with season. Boar sperm are extremely sensitive to chilling below 15°C thus extended boar semen is usually stored at a temperature of 17-18°C. As this temperature is difficult to maintain, especially during transport in the Prairie climate, fluctuations in temperature during shipping or storage that affect the quality of the extended semen can often go unnoticed. Developing the

ability to store liquid boar semen at 5°C would greatly improve the ability of Saskatchewan pork producers to import or export semen to and from their production units without affecting its quality.

Two of the extenders chosen for this study (Androhep®, X-Cell™) are the extenders most commonly used in the industry. The third extender (Mulberry III) is a new extender and it has been reported that adequate motility and fertility of liquid boar semen is maintained following storage in this extender at 5°C. Such results are limited to a few animals and have not been confirmed by any other study.

Project Objectives

The objectives of this study were:

1) To determine the effects of stepwise cooling combined with different incubation times at 17°C on the ability to store liquid boar semen at 5°C;
2) To determine the effect of three commercial extenders on the ability to store liquid boar semen at 5°C.

Methodology

A total of 18 ejaculates were collected from seven trained crossbred boars (Camborough x Canabrid; PIC) housed in individual boar crates. Individual semen samples were analyzed for volume, concentration, total and progressive motility, morphology and viability immediately after collection, prior to dilution. Individual ejaculates containing enough sperm for at least 24 doses were then split into three portions, each extended in one of the three extenders at 35°C, giving 8 doses per extender. Each dose contains 2.0×10^9 sperm in a total of 70 ml. One insemination dose from each of the three extenders was subjected to the following cooling treatments:

1. Stepwise cooling from 35 to 17°C over 180 min. Storage at 17°C. (Stepwise cooling consisted of placing the 35°C extended semen into consecutive water baths of 32, 29, 25, 22, 19 and 17°C. Semen was changed from one bath to the next every 30 min).
2. Stepwise cooling from 35 to 17°C over 180 min. Incubation at 17°C for 0 hr, transfer to 5°C cooler for storage.
3. Stepwise cooling from 35 to 17°C over 180 min. Incubation at 17°C for 4 hr, transfer to 5°C cooler for storage.
4. Stepwise cooling from 35 to 17°C over 180 min. Incubation at 17°C for 24 hr, transfer to 5°C cooler for storage.
5. Direct cooling from 35 to 17°C over 90 min. Storage at 17°C.
6. Direct cooling from 35 to 17°C over 90 min. Incubation at 17°C for 0 hr, transfer to 5°C cooler for storage.
7. Direct cooling from 35 to 17°C over 90 min. Incubation at 17°C for 4 hr, transfer to 5°C cooler for storage.
8. Direct cooling from 35 to 17°C over 90 min. Incubation at 17°C for 24 hr, transfer to 5°C cooler for storage.

Extended semen samples were evaluated for total and progressive motility, morphology and viability at 26, 48, 72, 96, 120 and 144 h after the initial achievement of 17°C.

The results expected from this project were:

1) The combination of stepwise cooling and 17°C incubation time that results in the greatest survival of boar sperm stored at 5°C;
2) The effect of the extender used on the ability of the various combinations of stepwise cooling and 17°C incubation time in improving the survival of boar sperm stored at 5°C.

Deviations

The first deviation was the timeline. The new research facility was initially stocked with mature boars in order to have the required number of back-up boars while the use of artificial insemination was being introduced. Training the mature boars, which were used only to natural mating, to service an artificial insemination dummy proved to be very difficult and took longer than anticipated. Typically, boars are trained to an artificial dummy at a young age without participating in natural mating.

The project as originally proposed was to evaluate 24 ejaculates from eight boars. However, due to the reduced size of the approved funding (from $23,080.54 to $13,080.00), it was possible to process and analyze only 18 ejaculates. This number still represents more than enough data to generate meaningful statistical conclusions.

The $600.00 in the budget heading "Other" has also been used to pay salaries. It was originally designated for publication costs, but for the same reason described above, it decided to put it towards the evaluation of the semen samples. This will not affect the distribution of the results of this project to the industry, as outlined in the original application, through the Technology Transfer program of Prairie Swine Centre Inc. The technology transfer program will play a central role in ensuring the results of the proposed research are communicated quickly and effectively to the Saskatchewan pork industry. Print publications including the Annual Research Report, updates in weekly marketing newsletters, trade publications and news releases will feature these results. Two articles featuring these results will be published in Prairie Swine Centre Inc.'s quarterly newsletter, 'Centred on Swine'. Electronic distribution via the Centre's Home Page will also be used. In addition, the Centre's scientists and management staff will personally appear at symposia, trade fairs, and producer round tables for face to face extension. The results will be shared with numerous industry networks (suppliers of feed, pharmaceuticals, breeding stock and veterinary services) in order to increase the number of individuals carrying the new technology to the primary pork producer. An abstract for presentation at the American Society of Animal Science meeting in Quebec in July 2002 will be submitted by the end of this January. A research article for submission for publication in a refereed journal is currently being prepared.

Please note that the amount spent on materials and supplies is in excess of the allotted $3,580.54 in the budget. This reflects both price increases in some of the supplies since the original application for this funding and an underestimation of the required amount of one of the disposable supplies.

Results

Statistical analysis of the extended semen samples was performed on each day of storage. There were significant main effects of extender, cooling rate/incubation time and day of storage on total and progressive motility. Table 1 shows the main effects of extender on total and progressive motility. The interaction between extender and cooling rate/incubation time was non-significant for both of these variables. For all six storage days, sperm stored in the Mulberry III extender had greater total motility than sperm stored in either the Androhep® or X-Cell™ extenders ($P < 0.05$). Progressive motility also varied among extenders. Sperm stored in Androhep® or X-Cell™ extenders had the greatest progressive motility on day 1 of storage but by day 2 and on, progressive motility was greatest for sperm stored in Androhep® and intermediate for those stored in X-Cell™ extender ($P < 0.05$). Progressive motility for sperm stored in Mulberry III extender was lowest for all 6 days of storage ($P < 0.05$). These results indicate that while all three extenders can maintain sperm motility over a period of six days, sperm stored in Androhep®, irregardless of cooling rate/incubation time, maintained the greatest total and progressive motility over time.

The main effects of cooling rate/incubation time on total and progressive motility are listed in Table 2. Total motility values were greater on days 2 to 6 of storage for sperm stored at 17°C, whether or not the cooling was done in a direct or stepwise fashion from 35 to 17°C (Treatments 1 and 5; $P < 0.05$). Storing at 5°C following 24 hr incubation at 17°C resulted in equivalent total motility on day 1 (Treatments 4 and 8), but by day 2 these values had decreased below those from sperm stored at 17°C ($P < 0.05$). Consistently lower total motility values were found in sperm stored at 5°C that had been incubated at 17°C for less than 24 hr (Treatments 2, 3, 6 and 7; $P < 0.05$). Similar to the total motility, progressive motility values on days 2 to 6 of storage were greatest for sperm stored at 17°C, whether or not the cooling was done in a direct or stepwise fashion from 35 to 17°C (Treatments 1 and 5; Table 2; $P < 0.05$). However, progressive motility values for sperm stored at 5°C, following direct cooling to 17°C and incubation at 17°C for 24 hr (Treatment 8), were equal to one or both of the treatments where sperm was stored at 17°C on all six days of storage ($P < 0.05$). Progressive motility values were lower for sperm stored at 5°C that had been incubated at 17°C for less than 24 hr (Treatments 2, 3, 6 and 7; $P < 0.05$). These results indicate that while storage at 17°C results in superior total motility, maintaining adequate progressive motility of extended boar sperm stored at 5°C is possible and the success is dependant upon a direct cooling rate to 17°C and 24 hr incubation time at 17°C.

There were significant main effects of cooling rate/incubation time and day of storage on normal morphology. The main effects of cooling rate/incubation time on normal morphology are listed in Table 3. There were no significant effects on day 1 of storage. For day 2 and on, all cooling rate/incubation time combinations had approximately equal normal morphology values, except for the lower values for those sperm incubated at 17°C for 0 hr prior to storage at 5°C (Treatments 2 and 6; $P < 0.05$). These results indicate that storing extended boar sperm at 5°C does not have a negative impact on morphology, provided that the sperm are incubated at 17°C for at least four hours prior to 5°C storage.

There were significant effects of extender, cooling rate/incubation time, day of storage and a significant interaction of extender by cooling rate/incubation time on viability. The

interaction effect of extender by cooling rate/incubation time on viability is listed in Table 4. For sperm extended in Androhep®, viability was greater on day 1 for those cooled stepwise to 17°C, incubated at 17°C for 4 hr prior to 5°C storage (Treatment 3) than those cooled directly to 17°C, incubated at 17°C for 0 hr prior to 5°C storage (Treatment 6; $P < 0.05$), but by day 2 of storage there were no differences among any of the cooling rate/incubation time treatments ($P > 0.05$). This is in contrast to sperm extended in X-Cell™ where viability values showed more variation, but no consistent differences among any of the cooling rate/incubation time treatments. However, sperm extended in X-Cell™, cooled directly to 17°C and incubated at 17°C for 0 hr prior to 5°C storage (Treatment 6) consistently had viability values that were among the lowest ($P > 0.05$). The viability results for sperm stored in the Mulberry III extender were similar to those for X-Cell™, with sperm cooled directly to 17°C and incubated at 17°C for 0 hr prior to 5°C storage again having viability values that were among the lowest (Treatment 6; $P > 0.05$). Similar to morphology, these results indicate that storing extended boar sperm at 5°C does not have a negative impact on viability, provided that the sperm are incubated at 17°C for at least four hours prior to 5°C storage.

These results indicate that it is possible to store extended liquid boar semen at 5°C with current, marketed extenders. While all three extenders can maintain sperm motility over a period of six days, sperm stored in Androhep®, irregardless of cooling rate/incubation time, maintained the greatest total and progressive motility over time. Storage of extended boar sperm at 17°C results in superior total motility, however, maintenance of adequate progressive motility of sperm stored at 5°C is possible and the success is dependant upon a direct cooling rate to 17°C and a 24 hr incubation at 17°C. Additionally, storing extended boar sperm at 5°C does not have a negative impact on morphology or viability, provided that the sperm are incubated at 17°C for at least four hours prior to 5°C storage.

In conclusion, storing extended boar sperm at 5°C while maintaining adequate progressive motility, morphology and viability is possible provided the sperm are cooled in a direct manner to 17°C and incubated for 24 hr at 17°C prior to lowering the temperature to 5°C. This procedure however, does not maintain total motility values that are as great as those for sperm stored at 17°C. Whether or not this statistical difference is of any practical significance will take an insemination trial to determine. An insemination trial to determine this, along with validating the other microscopy data from the most promising processing technique (cooling in a direct manner to 17°C, incubating for 24 hr at 17°C prior to storage at 5°C), would be very useful in ensuring that the laboratory data successfully predict the ultimate fertility data.

Personnel

Murray Pettitt is the principal investigator responsible for co-ordinating and overseeing the project. He spent a total of 155 hours on this project.

Eduardo Beltranena is the co-investigator responsible for this project. He spent a total of 12 hours on this project.

Amy Keefe was the Primary Research Technician performing the semen processing and analyses. She has spent a total of 303.85 hours on the project. Salary paid was $6926.86.

Danielle Bauche was a Research Technician performing viability and morphology analyses. She has spent a total of 20 hours on the project. Salary paid was $251.00.

Andre Palasz was a Research Assistant who performed viability and morphology analyses. He spent a total of 135.90 hours on the project. Salary paid was $1223.06.

Bob Morey was a stockperson assisting with the boars. He has spent a total of 4.0 hours on this project. Salary paid was $100.45.

Brian Thompson provided a statistical consult on the analysis of the results of this study. He spent 2.5 hours on this project. Fee paid was $137.50.

Equipment

An additional microscope was rented at a rate of $75.00/month. This was to allow multiple persons to evaluate the stained slides for morphology and viability of the extended semen, as this was the only way to complete the project by the reporting deadline.

Project Developed Materials

None have been developed at this time.

Project Photos

None taken at this time.

Acknowledgement

Acknowledgement of the Saskatchewan Department of Agriculture and Food's support of this project will be made at any future public presentation or in any publication of this work.

Final Budget

See attached

Table 1: Main effects of extender on total and progressive motility (%, lsmean ± sem)

Extender	Day of Storage					
	1	2	3	4	5	6
Total Motility[a]						
Androhep®	66.00a	64.20a	64.30a	64.92a	61.52a	56.82a
X-Cell™	66.10a	64.37a	66.52a	65.96a	62.24a	60.96b
Mulberry III	71.40b	67.03b	68.38b	68.81b	65.90b	59.37b
Progressive Motility						
Androhep®	60.12a	54.32a	43.47a	44.29a	39.57a	42.54a
X-Cell™	56.06a	43.72b	34.54b	31.62b	29.95b	32.12b
Mulberry III	30.85b	20.48c	14.43c	10.69c	7.36c	8.49c

[a]within variable and column, lsmeans with unlike superscripts significantly different ($P < 0.05$)

Table 2: Main effects of cooling rate/incubation time on total and progressive motility (%, lsmean ± sem)

Cooling Rate/ Incubation Time	Day of Storage					
	1	2	3	4	5	6
Total Motility[a]						
1*	78.93a	79.81a	82.84a	83.19a	80.69a	78.96a
2	54.77c	52.92b	52.42d	51.69b	50.06b	46.19b
3	60.08b	59.05c	59.41c	60.04c	56.59c	50.44b
4	78.37a	72.53d	73.19b	74.44d	68.00d	62.48c
5	79.94a	80.10a	82.17a	82.18a	78.21a	76.30a
6	48.97d	46.71e	49.44d	44.54e	43.67b	38.84d
7	63.19b	59.15c	59.02c	62.29c	57.30c	53.93b
8	78.39a	71.33d	72.72b	74.15d	71.23d	65.28c
Progressive Motility						
1	56.95a	48.08a	40.67a	33.84ac	32.04ac	36.70a
2	42.60bc	35.85bf	25.02b	26.32ab	21.76b	27.96bc
3	45.37bc	35.89bcf	24.55b	23.99bd	21.33b	23.67bd
4	51.07ac	40.65bdf	30.24b	27.98ade	23.28ab	25.42bd
5	55.57a	46.53ad	37.77ac	36.75c	33.81c	34.26ac
6	42.92bd	29.36ce	26.68b	26.94ad	24.57ab	23.30bd
7	47.74bc	35.69be	31.31bc	24.91bde	23.13b	22.84bd
8	49.84acd	43.99af	30.26bc	30.20acd	25.0[illegible]ab	27.56acd

[a]within variable and column, lsmeans with unlike superscripts significantly different ($P < 0.05$)

*Cooling Rate/Incubation Time:

1. Stepwise 35 - 17°C; store 17°C
2. Stepwise 35 - 17°C; incubate 17°C for 0 hr, store 5°C
3. Stepwise 35 - 17°C; incubate 17°C for 4 hr, store 5°C
4. Stepwise 35 - 17°C; incubate 17°C for 24 hr, store 5°C
5. Direct 35 - 17°C; store 17°C
6. Direct 35 - 17°C; incubate 17°C for 0 hr, store 5°C
7. Direct 35 - 17°C; incubate 17°C for 4 hr, store 5°C
8. Direct 35 - 17°C; incubate 17°C for 24 hr, store 5°C

Table 3: Main effects of cooling rate/incubation time on normal morphology (%, lsmean ± sem)

Cooling Rate/ Incubation Time	Day of Storage					
	1	2	3	4	5	6
Normal Morphology[a]						
1	85.79	79.93a	82.33a	84.38a	85.56a	81.44ab
2	83.12	76.92bc	77.38bc	77.61b	79.49bc	77.94ab
3	84.89	77.99ab	80.55ac	81.45a	82.52abc	81.20ab
4	82.98	79.18ac	80.69ac	83.48a	84.91ab	80.62ab
5	84.29	78.10ab	80.34ac	83.64a	83.72ab	81.34ab
6	83.17	74.09b	75.66b	77.71b	78.22c	76.80a
7	85.00	77.27ab	77.71ab	81.30a	81.33bc	81.23ab
8	84.90	80.09ab	80.40ac	82.68a	82.70ab	81.80b

[a]within column, lsmeans with unlike superscripts significantly different ($P < 0.05$)

*Cooling Rate/Incubation Time:

1. Stepwise 35 - 17°C; store 17°C
2. Stepwise 35 - 17°C; incubate 17°C for 0 hr, store 5°C
3. Stepwise 35 - 17°C; incubate 17°C for 4 hr, store 5°C
4. Stepwise 35 - 17°C; incubate 17°C for 24 hr, store 5°C
5. Direct 35 - 17°C; store 17°C
6. Direct 35 - 17°C; incubate 17°C for 0 hr, store 5°C
7. Direct 35 - 17°C; incubate 17°C for 4 hr, store 5°C
8. Direct 35 - 17°C; incubate 17°C for 24 hr, store 5°C

Table 4: Effect of extender x cooling rate/incubation time on viability (%, lsmean ± sem)

Extender x Cooling Rate/ Incubation Time	Day of Storage					
	1	2	3	4	5	6
Viability[a]						
A1*	84.04ab	76.48a	82.32a	84.40a	84.49a	75.82a
A2	78.75ab	78.39a	76.06a	75.86a	74.79a	74.55a
A3	87.09a	80.76a	81.95a	86.55a	83.64a	78.28a
A4	79.41ab	84.36a	81.00a	85.86a	77.93a	71.55a
A5	78.88ab	76.36a	81.19a	84.08a	80.26a	74.74a
A6	73.47b	76.25a	79.2a	75.18a	76.50a	70.93a
A7	81.00ab	80.74a	82.17a	83.11a	79.87a	75.40a
A8	82.40ab	84.09a	84.62a	77.76a	81.16a	79.95a
X1	85.13a	82.69a	86.05ab	81.28ab	83.53a	77.45abc
X2	74.68ab	74.00ab	72.93ab	73.67a	69.90bc	66.95a
X3	74.61bc	76.34ab	81.49ab	85.69b	81.82a	77.06abc
X4	79.49ac	79.22a	86.32a	83.00ab	83.08a	80.37bc
X5	79.34acd	83.54a	80.71ab	80.02ab	83.87a	73.26abc
X6	69.02bd	67.35b	73.13b	71.46a	64.03b	69.37ab
X7	78.61acd	79.17a	79.33ab	79.13ab	80.67ac	81.74c
X8	78.82acd	80.01a	82.82ab	80.00ab	82.33a	67.39a
M1	85.26a	78.96a	83.93a	80.92a	83.76a	76.58ab
M2	72.27bc	70.14ab	75.00ab	73.24a	68.59b	67.77a
M3	82.75ab	80.19a	80.04a	80.80a	83.10ac	78.13ab
M4	84.59ad	78.73a	82.52a	82.61a	82.07ac	80.09b
M5	74.81bde	81.51a	75.30ab	81.28a	77.65ab	73.68ab
M6	68.62ce	67.34b	66.22b	72.65a	72.11bc	68.06a
M7	76.21abe	77.97ab	74.49ab	80.27a	76.65ab	74.80ab
M8	78.29abe	77.13ab	74.53ab	80.43a	84.08a	75.12ab

[a]within column, lsmeans with unlike superscripts significantly different ($P < 0.05$)

*Extender: A = Androhep®; X = X-Cell™; M = Mulberry III

*Cooling Rate/Incubation Time: 1 = Stepwise 35 - 17°C; store 17°C. 2 = Stepwise 35 - 17°C; incubate 17°C for 0 hr, store 5°C. 3 = Stepwise 35 - 17°C; incubate 17°C for 4 hr, store 5°C. 4 = Stepwise 35 - 17°C; incubate 17°C for 24 hr, store 5°C. 5 = Direct 35 - 17°C; store 17°C. 6 = Direct 35 - 17°C; incubate 17°C for 0 hr, store 5°C. 7 = Direct 35 - 17°C; incubate 17°C for 4 hr, store 5°C. 8 = Direct 35 - 17°C; incubate 17°C for 24 hr, store 5°C.